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16. ABSTRACT

This report presents the results of two experiments performed at CERL in an effort to determine the suitability of a soil core microcosm as a screening tool under FIFRA. The soil core microcosm consisted of a 5 x 10 cm soil core removed intact from a field site and encased in PVC. In Experiment I, 0.25 lb/ac applications of ^{14}C-labeled dieldrin, methyl parathion and 2,4,5-T were examined, whereas 0.25, 0.50 and 1.0 lb/a applications of HCB were studied in Experiment II. Weekly leachates were analyzed for NO_3^{-1}, PO_4^{-1}, NH_3, Ca^{+2} and DOC as well as ^{14}C. The majority of the chemicals from both experiments found in the soil were in the top 2 cm. Extractable metabolites were detected for all but HCB. 2,4,5-T had practically no effect on any nutrient losses. NO_3^{-1} loss was impacted by dieldrin and methyl parathion. PO_4^{-3} was impacted by methyl parathion, 0.5 lb/a HCB and 1.0 lb/a HCB. NH_3 was impacted by the two upper levels of HCB. Calcium export was altered by methyl parathion and dieldrin. DOC was significantly impacted by methyl parathion and 1.0 lb/a HCB. Treatment levels for all chemicals were below normal application rates and did not really challenge the system.

It is possible to gain some assessment of chemical effect on a soil ecosystem, its fate and metabolism with the soil core microcosms.

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THE SOIL CORE MICROCOSM—

A POTENTIAL SCREENING TOOL

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major installations, one of which is the Corvallis Environmental Research Lboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake and stream systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report is a product of the Environmental Protection Agency's Alternative Chemicals Program, which in part seeks alternative methods for evaluating environmental impacts of both new and old pesticides. Screening techniques are essential in the registration and re-registration process required under FIFRA and for chemical testing under TSCA. Both regulatory agencies and chemical manufacturers will benefit by the use of test methodologies that provide the necessary data for safe and effective use of agricultural chemicals and other toxic substances in a timely and cost effective manner.

To this end we have examined a microcosm system which may meet those needs of both industry and government in avoiding unreasonable impact on man and his environment.

James C. McCarty
Acting Director, CERL

ABSTRACT

Two experiments have been performed to determine the suitability of a soil core microcosm (SCM) as a screening tool under FIFRA and TSCA. The SCM consisted of a 5- x 10-cm soil core removed intact from a field site and encased in PVC. Experiment I examined 0.25 lb/a applications of ^{14}C-labeled dieldrin, methyl parathion and 2,4,5-T. In Experiment II 0.25, 0.50 and 1.0 lb/a applications of HCB were studied. Weekly leachates were analyzed for nitrate (NO_3^{-1}), phosphate (PO_4^{-3}), ammonia (NH_3), calcium (Ca^{+2}) and dissolved organic carbon (DOC) as well as ^{14}C. Transport through the soil column and subsequent metabolism were followied via ^{14}C. The majority of the chemicals from both experiments found in the soil were in the top 2 cm. Extractable metabolites were detected for all but HCB. 2,4,5-T had no effect on any nutrient losses. Nitrate loss was increased by dieldrin and decreased by methyl parathion. Phosphate export was decreased by methyl parathion, 0.5 lb/a HCB and 1.0 lb/a HCB. Ammonia loss was decreased by the two highest levels of HCB. Calcium export was decreased by methyl parathion and increased by dieldrin. DOC was significantly decreased by methyl parathion and 1.0 lb/a HCB. Treatment levels were below normal application rates for all chemicals and did not really challenge the system.

These experiments demonstrate that it is possible to assess some effects of a chemical on a soil ecosystem and its fate simultaneously with the soil core microcosm.

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SECTION 1

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. Extraction of cores from field sites can be considered an "art", therefore, it can be expected that different technicians could produce different configurations of cores.

2. Inherent variation in natural ecosystems necessitates the use of at least 25 cores per treatment, assuming that some cores will be discarded during the equilibration period due to aberrant leaching rates and that three cores per treatment per week should be destructively analyzed for chemical movement. This will leave 9-12 cores for leaching and terminal ^{14}C analysis.

3. CO_2 measurements may be questionable due to high degree of intrinsic variation plus competition between plants and the chemical trapping agent for the CO_2 during photosynthesis.

4. The soil core permits the examination of effects on cycling of selected nutrients (phosphate, ammonia, calcium). Nitrate and DOC do not appear to be suitable parameters; nitrate may not be leached because of low availability in soil and whereas DOC measurements can be obscured by variations in plant and animal biomass between cores.

5. Fate, including transport and metabolism of a chemical may be followed throughout the soil system, although not with respect to air loss without considerable sampling apparatus.

In summary, it is possible to gain some assessment of the effect of a chemical on the soil ecosystem, its fate, transport and metabolism at a relatively low cost (approximately $40K/chemical).

RECOMMENDATIONS

Future work with soil core microcosms should include:

1. Continued evaluation of the nutrient parameters with emphasis on direct links to key ecosystem components or processes.

2. Refinement of the CO_2 sampling system to reduce variation and account for the competition of plants during the photosynthetic period.

3. Round-robin testing of the system with three or more laboratories examining the same chemicals.

4. Comparison of results with larger microcosms (e.g. CERL TMC).

5. Development of mathematical models with emphasis on nutrient cycling and microbial respiration.

SECTION 2

INTRODUCTION

TESTING REQUIREMENTS

Under the current guidelines of the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) as amended and the Toxic Substances Control Act (TSCA), chemical manufacturers are required to perform a variety of tests on the metabolism and mobility of a chemical in soil. For example, under FIFRA Section 163.62-8 of the "Proposed Guidelines for Registering Pesticides in the United States" requires data on aerobic and anaerobic soil metabolism reflecting the rate, type and degree of metabolism in at least three soils. In addition to transformation, information on the effects of the pesticide on microorganisms is required for such microbial processes as oxygen consumption, carbon dioxide evolution, nitrogen cycle reactions and measurement of enzyme activity for dehydrogenase or phosphatase. With respect to mobility (Section 163.62-9) data on the extent and rate of leaching, volatility, and adsorption/desorption are required (Johnson, 1978).

Similarily, under TSCA the guidelines for premanufacture testing tentatively recommend testing of adsorption of the chemical in soil and sediment as a means of assigning the chemical to one of four mobility classes (Section A-3.5) and of aerobic and anaerobic biodegradation to assess persistence in soil (Section A-4.5). Effects of microorganisms on metabolism of the chemical and effects of the chemical on the microorganisms as indicated by CO_2 evolution are also recommended (Sections A-4.54, A-4.55). Section C-3 further examines microbial effects using tests on organic matter decomposition and nitrogen and sulfur transformations (Muir, 1979). Generally these tests incorporate a variety of different testing apparatus and methods, necessitating different facilities and handling techniques. A single system capable of providing the needed data on metabolism, mobility, and microbial effects could expedite the testing and evaluation processes. Because such a system would combine the requirements of several tests, it would not necessarily be the optimum tool for all tests. Any single system attempting to provide data from several distinct tests would have to be viewed as a compromise providing reasonable although not necessarily the best data within its physical limitations. Industry and government must recognize its limitations and determine the criteria by which such a system could be appropriate and applicable to any testing scheme.

THE SOIL CORE MICROCOSM

Such a system may be a soil core microcosm (SCM) originally developed at Oakridge National Laboratory (Draggon, 1976, 1979; O'Neill et al., 1977; Jackson et al., 1977; EPA, 1977). The SCM concept developed around the theory

that an intact ecosystem or portion thereof could better represent a "real world" situation than a synthetic system so long as key processes could be adequately examined in the light of the inherent variability of a natural system (Ausmus et al., In Witt and Gillett, 1979). Early efforts centered around the loss of certain key nutrients (nitrate, phosphate, ammonia, calcium, DOC) via leachate as an indicator of community integrity and CO_2 production as an indicator of microbial respiration. More recent studies (Van Voris et al., 1978) have demonstrated within a controlled laboratory setting how these microcosm measurements can be used to evaluate complex relationships, such as that between ecosystem diversity and resistance/resilience of the ecosystem to pollutant impact.

A wide variety of terrestrial microcosm systems have been developed (Witt and Gillett, 1979), mostly as synthetic or constructed systems for evaluating the fate and effects of organic chemicals. The recent "Workshop on Terrestrial Microcosms" (Gillett and Witt, 1979) reviewed the state-of-the-art of terrestrial microcosm technology and found that current systems had a number of deficiencies which greatly limited their use as screening tools. The SCM appeared to be the most satisfactory for effects studies, but had not been tested with organics. The soil-litter ecosystem respirator (Lighthart and Bond, 1976) addressed more limited effects (decomposition) and also had been tested only with inorganics. The plant-soil system of Lichtenstein and coworkers (1974) had been tested with organic pesticides, but could not be expected to provide realistic effects for intact ecosystems. Larger systems (Cole et al., 1976; Gillett and Gile, 1976; Nash et al., 1977) appeared to have similar limitations, in addition to being less practical and more costly when used in a screening mode. General criticisms for all systems include: lack of inter-laboratory validation, lack of field verification of accuracy of impact predictions, deficiencies in simulating environmental conditions (air flows, temperature regimes), and deficiencies in criteria for acceptability (scaling criteria, degree of complexity, etc). Yet the conclusion was that terrestrial microcosm systems offered very real advantages and showed considerable promise in being able to deliver an integrated view of the fate and effects of a chemical.

Not all of the deficiencies in these systems can be explored. Because of its size, ease of replicability, adaptability to projected sites of impact, simplicity of approach, and relative low cost, the SCM has been selected for further evaluation. The experiments reported herein consider the applicability of the SCM to screening by combining portions of two types of protocols: studies of the fate (transport and transformation) of a chemical within the soil column through which water is percolating, and studies of the effects of the chemical on the soil community as evidenced by changes in micronutrient loss through the leachate and by changes in community respiration as evidenced by altered CO_2 production. Moreover, the emphasis in these studies is on synthetic organic chemicals used as pesticides or appearing as hazardous materials in the environment.

Among the purposes of these experiments are the need to compare costs of achieving a certain level of knowledge about a chemical's behavior in the environment, to compare the SCM technology with that of other systems, and to gain experience with the system by personnel other than the originators.

Indeed, work was needed to improve the technology *per se* and determine the extent to which the SCM could be standardized. The basic operating scheme is that suggested by Ausmus *et al.*, 1977 as reported in Gillett and Witt (Appendix B) 1979. This was modified by conversations with Ausmus, Draggon, Van Voris, Jackson and others regarding their experience with the system at Oak Ridge and during the IERL-Environmental Assessment testing of energy-related pollutants (EPA, 1977). The SCM protocol was then merged with the procedures of soil sampling, extraction and chromatographic analysis applied to other terrestrial systems (Cole *et al.*, 1976; Gile and Gillett, 1979a,b) for chemical fate. Comparisons can thus be made between these studies and those performed with other model ecosystems or in the field.

SECTION 3

METHODS AND MATERIALS

SOIL CORE MICROCOSM

The soil core microcosm consisted of a 5- x 10-cm soil core extracted intact from the field sampling site (a ryegrass pasture adjacent to CERL for Experiment I; Schmidt Farm for Experiment II). The cores were first trimmed of any extraneous material and then placed on a perforated polyethylene disk and encased in heat-shrinkable polyvinyl chloride (PVC), 1.5-mil thick, with a plasticizer content of 5-10%. The PVC was molded to the soil surface with 150°C air from a heat gun for approximately 20 seconds. Prior to shrinkage the cores were chilled by incubation at 5°C for 12 hr to minimize the heat stress on the microorganisms. Above ground vegetation was clipped at the onset of Experiment I and weekly thereafter. Vegetation on the cores in Experiment II was left intact.

The encased core was then cemented on a glass funnel with silicon rubber and fitted with a leachate collection flask (Fig. 1). The assembled SCM was then leached with 50 ml of standard reference rainwater (Lee, 1976), and cores which leached in less than 10 min or in more than 12 hours were rejected. The remaining SCM's were placed in racks in the Terrestrial Microcosm Chamber (TMC's; Gillett and Gile, 1976) or standard plant growth chambers, which permitted control of environmental temperature, light, and air flow. The air in each chamber in Experiment I was filtered through polyurethane foam pads (4.5- x 1.3-cm). In Experiment II the air was not analyzed for ^{14}C due to the configuration of the air systems in the growth chamber. The SCM's were maintained under a 16-hr daily light cycle with a temperature ranging from 27°C (day) to 16°C (night). The schedule of leaching and treatment was initiated on Day 0, as shown in Table 1.

For the next 28 days the cores were allowed to equilibrate with weekly leaching with enough standard rainwater to allow collection of 30 ml of leachate. The leachate was analyzed for nitrate, phosphate, ammonia, and calcium ions and for dissolved organic carbon to establish a baseline.

TREATMENT

After the equilibration period (4 weeks) each SCM was treated with 1 ml of xylene solution containing the pesticide or without amendment (control). In Experiment I, the treated SCM's received approximately 55 µg of ^{14}C-labeled chemical containing 0.5 µci of ^{14}C, applied at a rate equivalent to 0.25 lb/a (0.32 kg/ha) or approximately 0.20 ppm (wgt/wgt). Dieldrin (HEOD; 1,2,3,4,-10,10a-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-

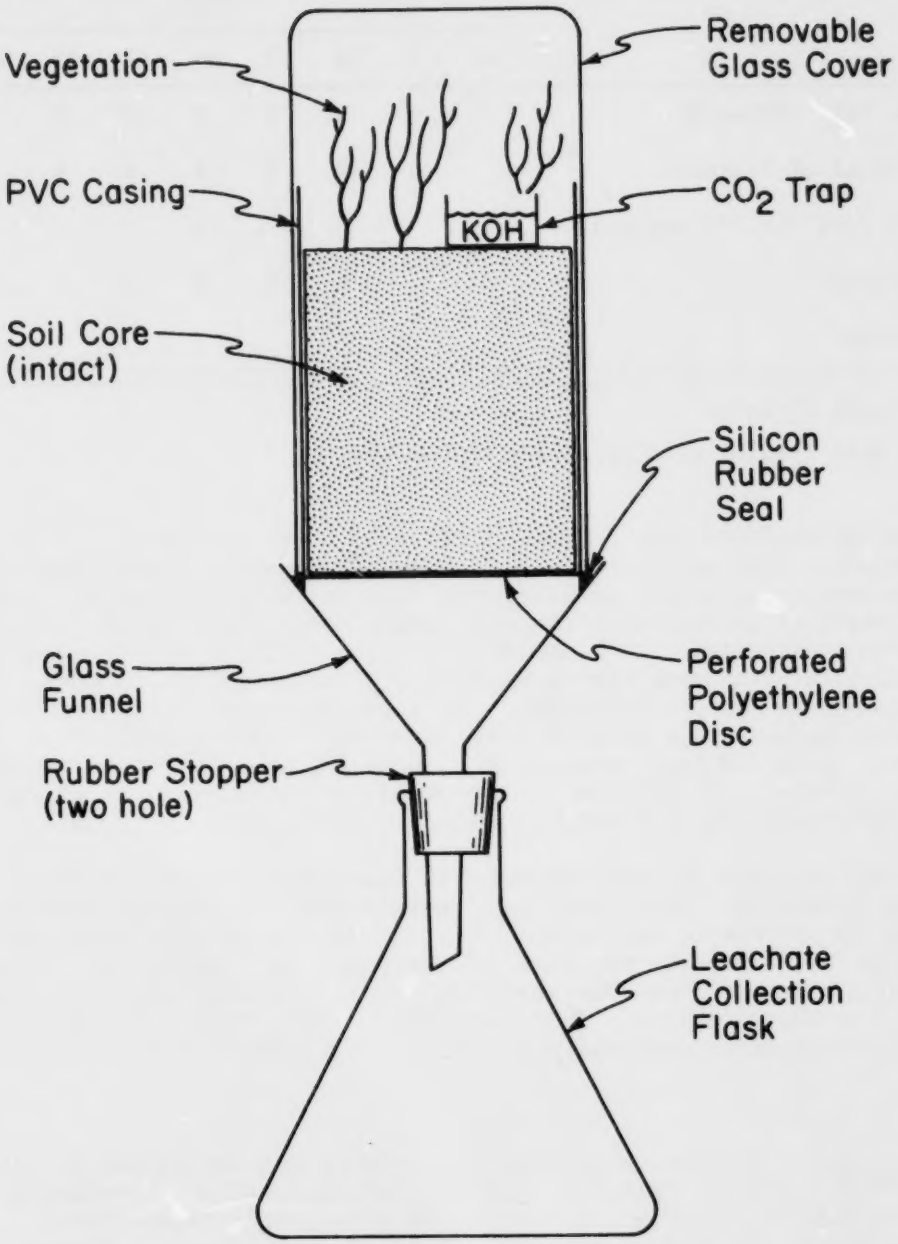

Figure 1. Soil core microcosm.

TABLE 1. EXPERIMENTAL PROTOCOL

	Day[b]								
	0	7	14	21	35	42	49	56	58
Add std. ref. rainwater	X	X	X	X	X	X	X	X	
Remove 30 ml of leachate		X	X	X	X	X	X	X	
Remove 1 core for ^{14}C analysis					X	X	X	X	
CO_2 analysis[a]			X	X	X	X	X	X	
Termination									X

[a] Experiment II only.

[b] Cores were treated with pesticide on Day 28.

dimethanonaphthalene) was obtained from Shell Chemical Company as an analytical standard (99% pure) and mixed with ^{14}C-labeled dieldrin (Amersham, 85% HEOD) to provide a xylene solution with 55 µg/ml at 85 mci/mmole. Similarly, methyl parathion (0,0-dimethyl-0-p-nitrophenyl phosphorothioate), octyl 2,4,5-trichlorophenoxyacetate, and hexachlorobenzene (HCB) solutions were prepared from analytical standards (Polyscience Corp.) and U-ring ^{14}C-labeled chemicals (New England Nuclear; 95% purity). In Experiment II, ^{14}C-labeled HCB solutions were applied at a rate of 0.25, 0.50, and 1.0 lbs/a equivalent in 1 ml of xylene. Each SCM thus received 55, 110 or 220 µg of ^{14}C-HCB and 0.5 µci of ^{14}C respectively. In addition to the positive control (xylene carrier), one set of SCM's received no treatment (negative control).

On Day 35, each SCM was leached with standard reference rainwater and the schedule resumed for other sampling. Weekly leachate samples were taken and analyzed for nutrients and total ^{14}C. At the end of each week, before the next water application, one core of each set was removed for analysis of ^{14}C-labeled chemical and the remaining cores (in Experiment II only) were checked for CO_2 evolution. Both experiments were terminated on Day 58, at which point all cores were analyzed for nutrient content and ^{14}C distribution.

ANALYSES

Nutrients: Nitrate, phosphate, and ammonia were determined by the phenolate method (cadmium reduction method) and ascorbic acid reduction method (Standard Methods, Rand et al., 1975) on a Technicon AutoAnalyzer. Calcium was determined via atomic absorption spectroscopy (Standard Methods, 1975). DOC was determined as total carbon by direct injection into an Oceanography International Carbonaceous Analyzer (Standard Methods, Rand et al., 1975).

CO_2 evolution: A 5-ml beaker containing 4 ml of 0.2 N KOH solution was carefully placed on the surface of the SCM at 0800 hrs, removed at 1600 hrs and back titrated with 0.1 N HCl solution. During the CO_2-trapping period, each SCM was covered with an inverted 150-ml beaker which was removed for all other operations.

Radiochemistry: Liquid scintillation spectrometry was used to determine ^{14}C levels in each sample. A volume of 1 ml of each leachate was checked immediately by direct addition to the scintillation cocktail (both for analysis and for worker protection regarding subsequent handling during chemical analyses). Soil extracts were checked for total activity then chromatographed on 250-μ silica gel G plates using a hexane:ether (1:1) solvent system. Materials were located by radioautography, extracted from the TLC plates, and analyzed. Metabolites were tentatively identified by comparative R_f values. Residual radioactivity after extraction of soil segments with KCl, hexane: isopropanol (3:2), or acetone was determined by combustion of the dried soil sample in a Packard Oxidizer and collection of the $^{14}CO_2$.

Termination (harvest) procedures: When a SCM was terminated, the core was stripped of the PVC coat (which was extracted with ethyl acetate) and sectioned into three segments approximately 2-, 3-, and 5-cm deep. The sections were extracted with 200 ml of 0.1 M KCl solution followed by aspiration of residual liquid. Each segment was freed of plant material then extracted in a Waring blender with one of the series of organic solvents and water to determine ^{14}C distribution of the parent chemical and any metabolites. The plant material was similarly extracted. Only extracts containing 10^3 dpm or greater were subjected to TLC, permitting ready detection of any extractable metabolites at the 1% level. Air filters were extracted with ethyl acetate and the extract counted.

QUALITY ASSURANCE

See Appendix C for Quality Assurance procedure.

DATA HANDLING

The results of each analysis were stored in a Control Data Corporation 3300 computer and accessed by a program which yielded the following computed values: total ^{14}C mass balance per SCM; fraction of radioactivity as equiv. of parent in each SCM section as parent, metabolites (including origin material and aqueous extracts which were not chromatographed), and bound residue; total nutrient content, weekly elution rate, and terminal nutrient residue for nitrate, ammonia, phosphate, cadmium, and DOC; and CO_2 evolution rate as a weekly 8-hr value. Leachate data were considered to be log-normally distributed. Statistical significance was determined by the methods of Snedecor and Cochran (1969), using Student's t test.

SECTION 4

RESULTS AND DISCUSSION

EXPERIMENT I [dieldrin (HEOD), methyl parathion (MP), 2,4,5-T (T)].

Mass Balance and Distribution

As indicated in Table 2, total recovery of ^{14}C varied substantially with the chemical under consideration. Values ranged from an average of 43.1% for methyl parathion to an average of 78.2% for dieldrin. The majority of the carbon-14, irrespective of the chemical, was found in the soil, with plants having the next highest level. Overall recovery of ^{14}C from each chemical from the soil core was generally lower than average recoveries from the CERL Terrestrial Microcosm Chamber (Gile and Gillett, 1979a,b; unpublished data). This lower accountability is not likely due to differences in the techniques relative to the media examined directly (e.g., soil, plants, leachate), as the coefficients of variation are typical for analyses of biological materials in both experimental regimes. Rather, the material lost to the air (intact, by volatilization of parent or metabolites, or as $^{14}CO_2$) would appear to be the likely route of the unaccounted mass balance. This could differ by virtue of: the differing "rainfall" regimes used, which might cause more of the chemical to be lost by displacement phenomena (Spencer and Cliath, 1969); the lack of plant cover (as compared to other terrestrial microcosm systems), decreasing the "still air layer" above the soil, and thus increasing exchange with air flow; the different physical configuration and greatly increased "edge" of the SCM as compared to the TMC; or the increased metabolism of the chemicals to $^{14}CO_2$. The last possibility seems most likely, since the proportion of material recovered as metabolite(s) and bound residue in the soil is much greater than observed in earlier experiments in the TMC. This would suggest that rates of degradation in intact ecosystems might be higher than those realized in synthetic systems, in agreement with observations by Paris (R. Lassiter, 1979) for soil/sediment/water enrichment cultures in pesticide degradation.

The majority of the applied chemicals recovered from the soil were found in the top segment (2-cm deep), as shown in Table 3. Each chemical behaved differently, however, and this test demonstrates how the SCM might yield valuable information. Methyl parathion and 2,4,5-T were found mostly as bound residues, whereas HEOD remained as intact parent. 2,4,5-T was more readily bound or assimilated at subsurface levels than HEOD or MP; ^{14}C from T also appeared in greater proportions in the leachate (Table 2 and Fig. 2). Clearly, T was the most mobile and least stable in this soil environment, while HEOD was very stable and not very mobile; MP more closely resembled HEOD in mobility and T in stability.

TABLE 2. ^{14}C ACTIVITY AT TERMINATION (% OF APPLIED)[a]

Component	Chemical		
	Methyl Parathion	2,4,5,-T	HEOD
Soil	30.0 [0.29[d]]	41.1 [0.24]	60.9 [0.15]
Plant	8.4 [0.51]	6.1 [0.59]	14.3 [0.49]
Leachate	0.5 [1.02]	2.5 [0.89]	0.2 [0.21]
Soil Case[b]	2.6 [0.79]	1.3 [0.79]	2.5 [0.55]
Air Filter[c]	0.6 -------	0.4 ------	1.0 ------
Total	43.1 -------	51.4 ------	78.2 ------

[a] Mean of 8 cores for methyl parathion; 9 for 2,4,5-T; and 12 for HEOD.

[b] PVC heat-shrinkable tubing and masking tape.

[c] Composite from a single filter apparatus/treatment.

[d] Coefficient of variation.

TABLE 3. PROFILE OF ^{14}C ACTIVITY IN SOIL AT TERMINATION (% OF TOTAL ^{14}C APPLIED)[a]

Level	P[b]	M[c]	B[d]	Total
		Methyl Parathion		
Top 2 cm	1.0 [0.14][e]	1.1 [0.14]	24.8 [0.39]	26.9 [0.36]
3-5 cm	0.6 -------	0.6 ------	0.73 [0.59]	2.0 [0.24]
6-10 cm	0.5 -------	0.5 ------	0.64 [0.42]	1.6 [0.16]
		2,4,5-T		
Top 2 cm	2.1 [0.28]	3.4 [0.25]	29.0 [0.32]	34.5 [0.26]
3-5 cm	1.2 [0.45]	1.0 [0.63]	2.9 [0.71]	5.1 [0.52]
6-10 cm	0.5 [0.47]	1.3 [0.03]	1.5 [0.54]	3.3 [0.38]
		HEOD		
Top 2 cm	43.6 [0.21]	4.4 [0.48]	3.8 [0.30]	51.8 [0.19]
3-5 cm	6.5 [0.50]	0.4 [1.2]	0.5 [0.46]	7.4 [0.52]
6-10 cm	1.7 [0.19[	0.0 ------	0.5 [0.12]	1.9 [0.16]

[a] Mean of 8 cores for methyl parathion; 9 for 2,4,5-T; and 12 for HEOD.

[b] Extractable parent.

[c] Extractable metabolites.

[d] Bound residues.

[e] Coefficient of variation.

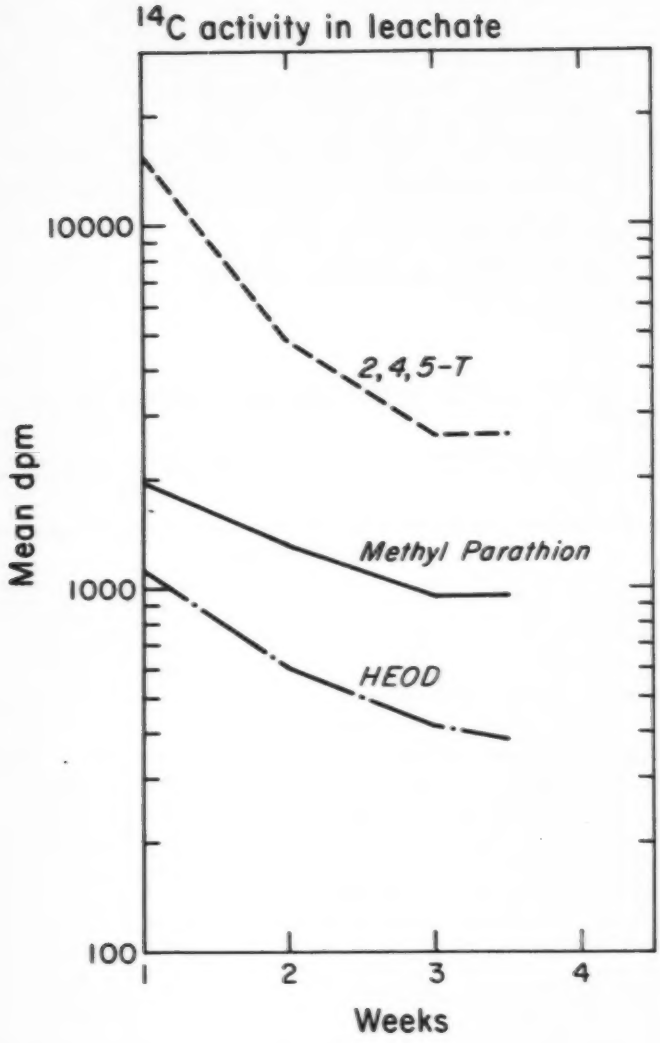

Figure 2. Mean ^{14}C activity in leachate for Experiment I.

Movement of the chemicals through the SCM is controlled by the interaction of several properties: volatility and fugacity; solubility; biodegradation rates; and adsorption/desorption rates and equilibria. Difference between SCM's might be due to organic matter content, bulk density (or its inverse, the degree of aeration of the soil by capillary channels), and the nature of biota present (since the small portion of rhizosphere present would be associated with only limited plant species). In spite of great intrinsic variability between SCM's regarding flow rate, for example, the results indicate that considerable consistency can be achieved. Prediction of the outcome should eventually be possible, based upon the physical/biochemical properties of the test chemical. Sorption phenomena have been related to octanol/water partition coefficient and organic matter content of the soil (R. Lassiter, 1979); volatility (from water), solubility, and partition coefficient also have been related (Chiou et al., In Witt and Gillett, 1979; Metcalf et al., 1979). Given the properties of these three test chemicals shown in Table 4, the performance of the SCM reveals the interactions fairly well in intercomparisons.

TABLE 4. PHYSICAL PROPERTIES OF TEST MATERIALS

Property	Methyl Parathion	2,4,5-T	HEOD
M.P.[a]	36°C	158°C	175°C
Vapor pressure[a]	9.7×10^{-6} (20°C)	1×10^{-2} (20°C)	7.78×10^{-7} (25°C)
Solubility[a]	55-60 (25°C)	278 ppm (25°C)	0.05 ppm
O/W partition[b]	2.8×10^{3}	9.2×10^{2}	3.9×10^{5}
Adsorption K[b]	5.6×10^{2}	2.2×10^{2}	3.4×10^{4}

[a] Spencer, 1973.

[b] Chiou, 1979.

O/W partition coefficient estimated from solubility.

Adsorption K calculations based only on organic matter weight in soil, ignoring the contribution of sorption by other constituents.

Movement throughout the soil core corresponds to the vapor pressure and solubility of the chemicals. Although both were extensively degraded in the soil, more methyl parathion was lost in the air due to a higher vapor pressure, whereas relatively more 2,4,5-T left the core via the leachate due to a higher solubility in water. With a low biodegradation rate, water solubility and vapor pressure more dieldrin would be expected to remain in the soil. Neither the leachate or air samples were analyzed by TLC, therefore the data reflect only ^{14}C activity.

Two metabolites of methyl parathion were detected in the soil. When their Rf's were compared to those of known metabolites of methyl parathion it appeared as though amino-methylparathion and p-nitrophenol were the major metabolites. Two metabolites of 2,4,5-T with Rf's similar to those of unidentified metabolites detected by Metcalf et al. (1979) were also detected but no attempt was made to identify them. The metabolites of HEOD, although unidentified, appear to have Rf's similar to those detected in the CERL TMC.

EFFECTS ON NUTRIENT LOSSES

The coefficients of variation calculated for the five nutrients examined (NO_3^{-1}, PO_4^{-3}, NH_3, Ca^{+2}, DOC) all fall within the range common to biological systems; however irrespective of treatment, PO_4^{-3}, NH_3 and Ca^{+2} appear to be much less subject to intrinsic variation at any given time and between different cores (Table 5).

TABLE 5. COEFFICIENT OF VARIATION (CV). EXPERIMENT I[a]

Parameter	CV
NO_3^{-1}	0.35
PO_4^{-3}	0.18
NH_3	0.13
Ca^{+2}	0.15
DOC	0.33

[a] Experiment I: methyl parathion, 2,4,5-T and HEOD.

Figure 3 depicts the loss of the five nutrients via the leachate over the course of the experiment. Of the treatments methyl parathion and dieldrin affected NO_3^{-1} loss relative to the control. Despite an initial increase in NO_3^{-1} loss the dieldrin treatment became not significantly different from the control within two weeks. Methyl parathion initially increased loss of NO_3^{-1}, but quickly recovered to produce significantly reduced NO_3^{-1} losses at the 90% confidence level. While all three chemicals appear to have reduced PO_4^{-3} export, only methyl parathion had a significant effect at the 90% level. NH_3 export in the leachate was not significantly influenced by any of the chemicals. Both dieldrin and methyl parathion significantly ($P \geq 0.1$) altered Ca^{+2} export with methyl parathion decreasing export while dieldrin slightly increased export. DOC levels in the leachate were only significantly impacted (at the 90% level) by methyl parathion which reduced export. Contrary to previous studies an increase in coefficient of variation does not appear to be consistently associated with a significant effect on nutrient loss (Table 6). C.V.'s remain relatively constant indicating the reliability of the post-treatment measurements.

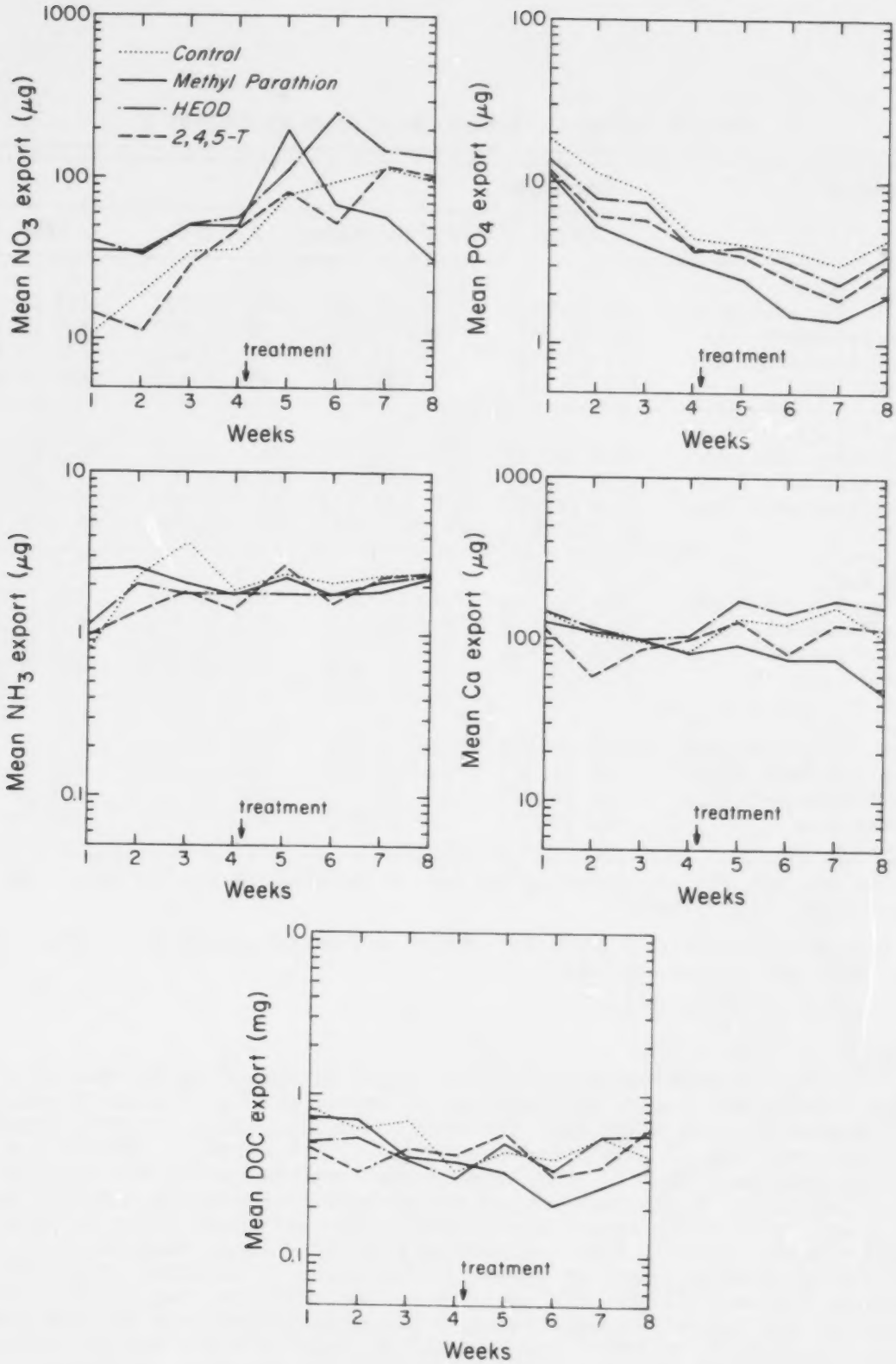

Figure 3. Mean nutrient levels in leachate for Experiment I.

TABLE 6. CUMULATIVE NUTRIENT LOSS IN EXPERIMENT I

Nutrient[a,b]	Treatment			
	Control	Methyl Parathion	2,4,5-T	HEOD
NO_3^{-1} (µg)				
Initially available	903 [0.89][c]	1344 [0.66]	1290 [0.26]	1464 [0.54]
Pre-treatment loss	105 [0.89]	105 [0.81]	105 [0.69]	209 [0.76]
Post-treatment loss	167 [0.65]	397 [0.84]	342 [0.60]	678 [0.75]
Total loss	272 [0.73]	503 [0.80]	448 [0.55]	887 [0.66]
PO_4^{-3} (µg)				
Initially available	98.5 [0.12]	113.6 [0.23]	96.4 [0.17]	98.5 [0.16]
Pre-treatment loss	25.0 [0.44]	42.4 [0.43]	25.8 [0.35]	33 [0.35]
Post-treatment loss	11.4 [0.61]	15 [0.61]	11.1 [0.58]	13.2 [0.39]
Total loss	36.5 [0.44]	57.8 [0.45]	37.1 [0.40]	46.1 [0.29]
NH_3 (µg)				
Initially available	1125 [0.32]	1749 [0.37]	1504 [0.38]	1400 [0.45]
Pre-treatment loss	6.5 [0.34]	8.4 [0.42]	5.1 [0.19]	6.5 [0.52]
Post-treatment loss	8.0 [0.29]	9.1 [0.16]	8.9 [0.28]	8.1 [0.12]
Total loss	14.5 [0.27]	17.5 [0.24]	14.0 [0.20]	14.5 [0.27]
Ca^{+2} (µg)				
Initially available	1862 [0.21]	1354 [0.46]	1231 [0.30]	1612 [0.33]
Pre-treatment loss	425 [0.60]	449 [0.63]	363 [0.46]	497 [0.47]
Post-treatment loss	236 [0.49]	514 [0.71]	449 [0.46]	666 [0.50]
Total loss	662 [0.55]	963 [0.65]	812 [0.43]	1162 [0.46]

[a] The use of a KCl extraction on the core at termination did not permit DOC analysis.

[b] Mean total nutrient; 8 cores for control and methyl parathion; 9 cores for 2,4,5-T; and 12 cores for HEOD.

[c] Coefficient of variation.

Of the three pesticide treatments, methyl parathion had the most significant impact, which would be judged to be largely beneficial since it resulted in greater retention of soil nutrients. Dieldrin had a slightly negative effect, resulting in some increased losses of Ca^{+2} and NO_3^{-1}, whereas 2,4,5-T had no detectable impact. Half-life for the chemical and the effect appear to have no direct relationship, since methyl parathion with the shortest half-life ($t_{\frac{1}{2}}$ = 25 days) seemed to have the most significant effect on nutrient loss. Half-lives of 2,4,5-T and HEOD were 30 and 67 days, respectively. Each of these, however, must be viewed in the light of possible impact of the solvent (xylene), since this would have dominated the comparisons. Thus, although the initial increase in NO_3^{-1} export associated with the HEOD treatment resulted in a 30% higher cumulative loss in comparison to controls, controls lost 30% of their available NO_3^{-1} in the leachate. Furthermore, the

methyl parathion treatment resulted in an increase in NO_3^{-1} export even though that loss was rapidly reversed and the rate of loss became lower than the control rate.

None of the treatments led to severe or irreversible displacements of any of the nutrients. The concentrations applied were at or below that recommended for actual use of these pesticides and therefore did not really challenge the system.

EXPERIMENT II [Hexachlorobenzene (HCB)]

Mass Balance and Distribution

Recovery of ^{14}C ranged from 33.6% at the 1 lb/a treatment to 43.3% at the 0.25 lb/a treatment (Table 7). The inverse relationship between the amount applied and that recovered probably reflects a saturation of the small volume of soil with HCB and a resultant volatilization of the ^{14}C material. In contrast to the first experiment, plants contain the majority of ^{14}C recovered even though the HCB was applied to the soil. One probable explanation is the greater abundance of plant biomass in Experiment II, since above ground vegetation was removed in Experiment I. No ^{14}C was detected in any of the leachate analyzed, which is not unexpected for HCB (< 0.02 µg/l solubility in water).

TABLE 7. ^{14}C ACTIVITY OF TERMINATION (% OF APPLIED)[a]

Component	Chemical		
HCB	0.25 lb/a HCB	0.50 lb/a HCB	1.0 lb/a
Soil	20.0 [0.32][c]	17.3 [0.30]	12.9 [0.30]
Plant	22.1 [0.42]	22.0 [0.39]	19.8 [0.30]
Leachate	0	0	0
Soil Case[b]	1.2 [0.37]	1.3 [1.07]	0.9 [0.27]
Total	43.3	40.6	33.6

[a] Mean of 15 cores for all treatments of HCB.

[b] PVC heat shrinkable tubing and masking tape.

[c] Coefficient of variation.

The majority of ^{14}C material recovered from the soil was in the top 2-cm layer and the results were quite consistent between the treatment levels (Table 8). No metabolites were detected, but bound residues tended to exceed free HCB to a greater extent with increasing depth in the soil profile, suggesting somewhat greater mobility of HCB metabolites. In earlier work with HCB (Gile and Gillett, 1979b), in which a 25-cm deep synthetic soil medium was used, extractable parent predominated in the first 10 cm of soil. Below that

TABLE 8. PROFILE OF ^{14}C ACTIVITY IN SOIL AT TERMINATION (% OF TOTAL ^{14}C APPLIED)[a]

	P[b]	M[c]	B[d]	Total
Level		0.25 lb/a HCB		
Top 2 cm	9.7 [0.45][e]	0	8.4 [0.35]	18.1 [0.34]
3-5 cm	0.6 [0.75]	0	0.7 [0.52]	1.3 [0.61]
6-10 cm	0.2 [0.41]	0	0.4 [0.14]	0.6 [0.18]
		0.50 lb/a HCB		
Top 2 cm	7.0 [0.45]	0	8.3 [0.39]	15.3 [0.32]
3-5 cm	0.4 [0.79]	0	0.7 [0.49]	1.1 [0.59]
6-10 cm	0.2 [0.41]	0	0.6 [0.34]	0.8 [0.32]
		1.0 lb/a HCB		
Top 2 cm	5.9 [0.64]	0	5.5 [0.25]	11.4 [0.42]
3-5 cm	0.4 [0.51]	0	0.6 [0.52]	1.0 [0.46]
6-10 cm	0.1 [0.32]	0	0.4 [0.15]	0.5 [0.14]

[a] Mean of 15 cores for all treatments.

[b] Extractable parent.

[c] Extractable metabolites.

[e] Coefficient of Variation.

depth HCB was present exclusively as extractable metabolites and bound residues.

As shown in Tables 9 and 10, most of the material remaining in the plants was unchanged parent HCB, with no detectable metabolites and an amount of bound residue proportional to the dose applied to the soil. An identical pattern was observed when HCB was followed in a larger system (Gile and Gillett, 1979b). Plant uptake responded nonlinearly, increasing less proportionately than the dose applied to the soil.

EFFECTS ON NUTRIENT LOSSES AND CO_2

Some NO_3^{-1} was detected in the soil, although none was leached from any of the treatments, including the control. The coefficients of variation for the four nutrients examined (PO_4^{-3}, NH_3, Ca^{+2}, DOC) generally exhibited some improvement in comparison to Experiment I (Table 11). DOC again appeared most variable, probably due to the high degree of variability in the amount of plant and animal biomass associated with individual cores.

Figure 4 depicts the loss of PO_4^{-3}, NH_3, Ca^{+2} and DOC from the soil core via the leachate throughout the course of the experiment. No effect on PO_4^{-3}

TABLE 9. PROFILE OF ^{14}C ACTIVITY IN PLANTS AT TERMINATION (% OF TOTAL ^{14}C APPLIED)[a]

	0.25 lb/a	0.50 lb/a	1.0 lb/a
P[b]	20.0	19.8	12.9
M[c]	0	0	0
B[d]	2.1	2.2	1.9

[a] Mean of 15 cores for all HCB treatments.

[b] Extractable parent.

[c] Extractable metabolites.

[d] Bound residues.

TABLE 10. PPM OF HCB AND BOUND RESIDUES IN PLANTS

	0.25 lb/a	0.50 lb/a	1.0 lb/a
P[a]	0.55	1.08	1.38
B[b]	0.06	0.12	0.20

TABLE 11. COEFFICIENTS OF VARIATION (CV). EXPERIMENT II[a]

Parameter[b]	C.V.
PO_4^{-3}	0.10
NH_3	0.15
Ca^{+2}	0.07
DOC	0.24

[a] Experiment II: 0.25, 0.50 and 1.0 lb/a HCB.

[b] NO_3^{-1} levels in leachate below detection limits.

levels was observed for the xylene carrier alone or with the 0.25 lb/a equivalent HCB. A significant effect at the 95% level for the 0.5 lb/a and at the 90% level for the 1.0 lb/a treatment was observed, with both treatments reducing PO_4^{-3} export. NH_3 loss was reduced by the xylene treatment at the 90% level and by the 0.50 and 1.0 lb/a HCB treatment at the 95% confidence level. Except for a reduction of Ca^{+2} loss by the xylene carrier none of the HCB treatments had a significant effect at the 90% level. Only the 1.0 lb/a treatment of HCB reduced the loss of DOC via the leachate at the 90% level.

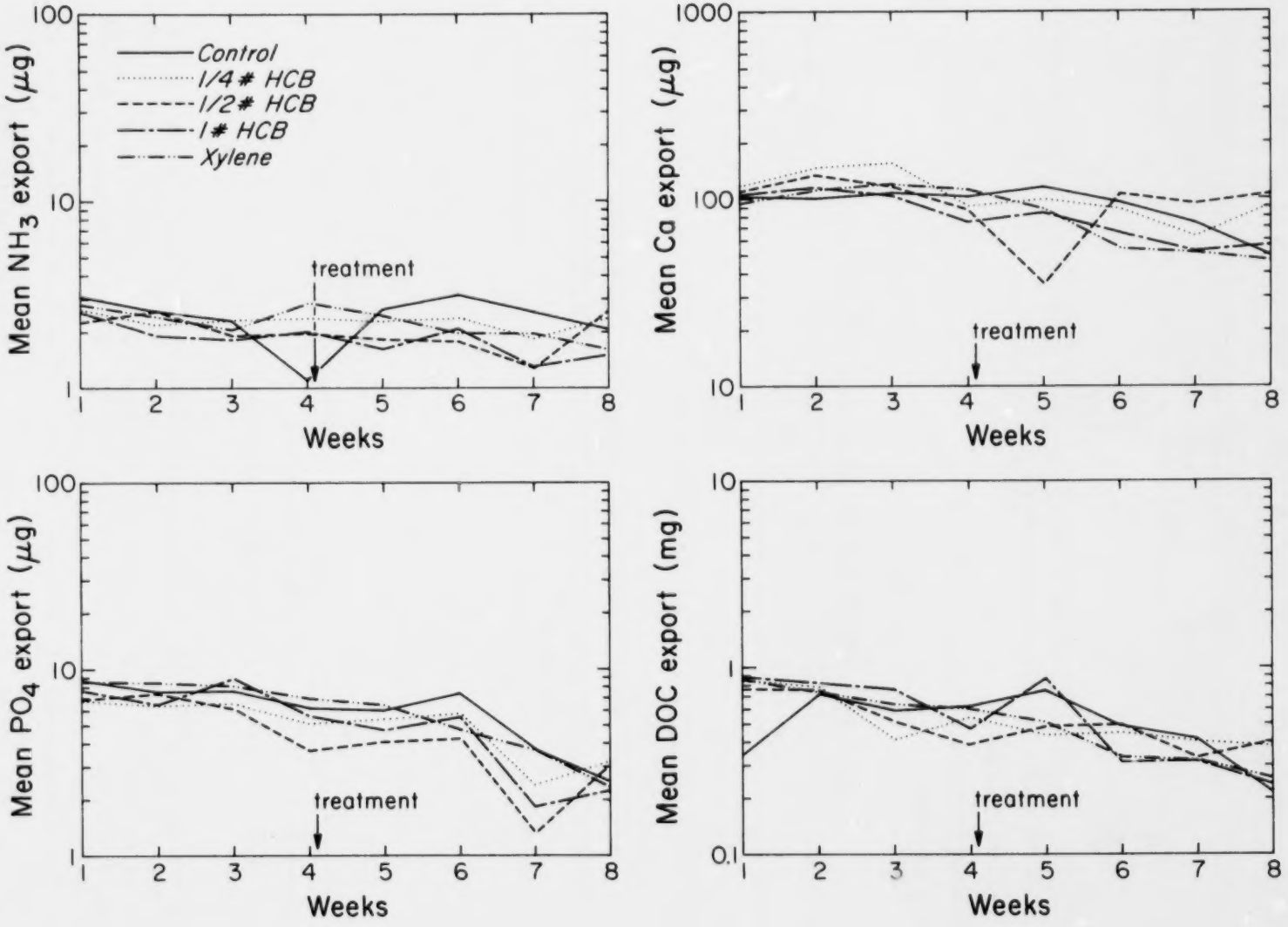

Figure 4. Mean nutrient levels in leachate for Experiment II.

There appears to be a reduction in the amount of CO_2 evolved with all levels of HCB and xylene alone followed by a temporary increase in CO_2 evolution and then another decline for the HCB treatments (Fig. 5).

As with Experiment I none of the treatment levels severely affected the soil core. Normally HCB has been applied as a fungicide at much higher levels and therefore should be tested at 1, 10 and 100 lb/a equivalents to determine the effects.

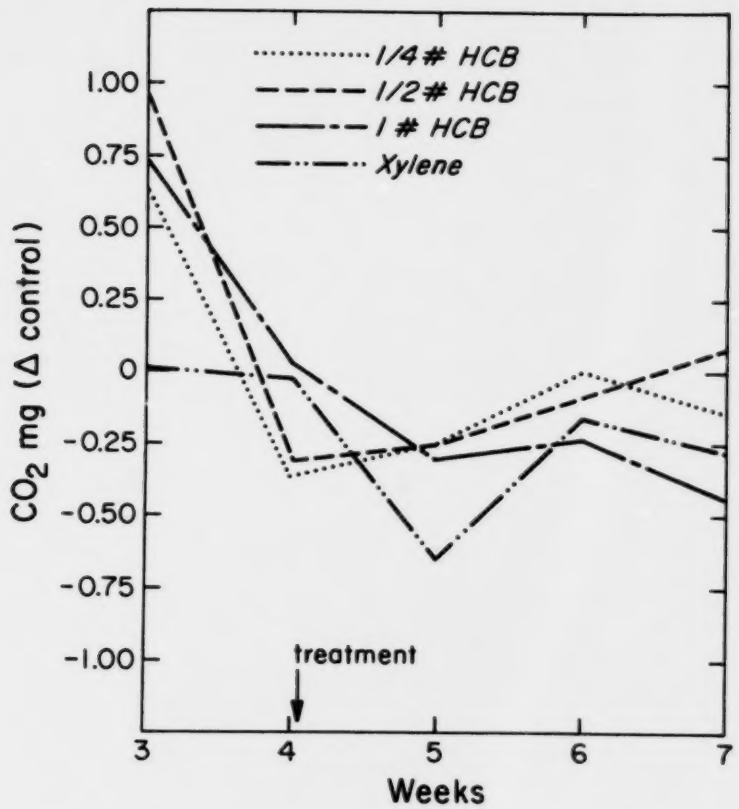

Figure 5. Mean CO_2 evolution for Experiment II.

SECTION 5

OVERVIEW

In these two experiments, which varied slightly from the suggested protocol (Gillett and Witt, 1979), an attempt was made to determine if fate and effects of a chemical could be evaluated simultaneously in a single test and, if so, could data be obtained more cost-effectively.

In terms of chemical fate, there were few surprises. More HEOD metabolites were recovered than in any previous tests, but the small fraction of HCB metabolites found in other experiments (Gile and Gillett, 1979b; Metcalf, 1979) was absent. Soil degradation rates and volatility losses were confounded, at least partially, by the interim sampling techniques and inadequate air monitoring. The analytical techniques employed radiometry (requiring ^{14}C-labeled chemicals and liquid scintillation apparatus), but could be adapted to standard analytical techniques to the extent that the methods were sensitive and available. This is less a limitation for pesticide registration, where requirements are such that radio-labeled chemicals may be involved in a variety of necessary studies, than for screening of toxic substances. However, only radiometry can reveal the extent of bound residues in soil or plant and animal tissues. Otherwise, radiometry compares favorably for the determination of parent compound in relation to gas-liquid chromatography with regard to sensitivity, specificity, and cost. Radiometry has decided advantages over other methods regarding metabolite detection, particularly as a screen.

The main difficulty is the volume of the sample load (and associated quality assurance). Experiment I involved the determination of parent and metabolites by TLC and of bound residues by combustion of over 100 samples, and Experiment II involved over 150 samples. Intermediate sampling of single soil cores proved fruitless, as at least 3 or 4 SCM's must be analyzed for statistical significance. The chemical fate work cost approximately 2 man-months of skilled chemistry technician time and 4 man-months of semi-skilled support. This level of effort seems necessary to overcome the inherent variability of the soil cores, even when they were collected from a single m^2 area.

Performing soil studies on three different types of soil at four levels with 12 cores at termination and three cores at each weekly date (pre-treatment, 1st, 2nd weeks after treatment) plus positive (carrier) and negative (no treatment) controls would require analysis of approximately 400 cores (1200 samples) and over 2000 samples of leachate. If an adequate air sampling system, such as a polyurethane plug, were added and sampled at weekly intervals after treatment, an additional 225 analyses would be required. If metabolites were identified or bound residues were further pursued, a substantial level of effort would be required, but that would be beyond the screening

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level envisioned for this test. As set forth above, such a test would cost approximately $40,000/chemical, to yield the following information:

* Estimate of overall half-life of chemical applied to three different soil types.

* Estimate of leachability (qualitative) in three soil types.

* Quantitative estimate of soil volatility under this moisture regime.

* Qualitative picture of metabolites and bound residue and estimate of mass balance by all known loss routes, with information on rates of transformation and movement in soil.

* Detection of irreversible change in terms of soil respiration or nutrient loss.

* Estimate of possible reversible impacts on soil respiration and nutrient loss.

As more experience is gained with this testing procedure, the "estimates" given above can be associated with more specific criteria of performance. These would be vital to efficient use of the SCM as a screening tool. Recommendations (Gillett and Witt, 1979), that application of microcosms be accompanied by an intensive effort to establish evaluative and predictive models, would greatly improve the utility of the SCM, if followed.

Use of the SCM to estimate the impact of a chemical on a simple soil community has solid support (Ausmus et al., reported in Gillett and Witt, 1979; O'Neill et al., 1977). If micro- and macronutrient losses are severe and irreversible, the stability of an unmanaged ecosystem would be threatened. A managed ecosystem (e.g., an agricultural or urban setting) would require some greater level of resource allocation to maintain productivity or other utility. Important questions revolve around the extent of irreversibility and the impact of "transient" (on a geological timescale) changes. Because of the attenuation of processes in the soil and the slow rate of change, the soil community seems to be relatively more resistent and resilient than other ecosystems. Moreover, any screening test should be accomplished in a much shorter time than these SCM experiments, but the time interval used is not even a significant fraction of the growing season.

The use of microcosm systems, such as the SCM, may be at an initial level of testing to provide basic data on the chemodynamics of a chemical or its effect on a general process. Alternatively, it may be used to confirm predictions or projections of fate and effects based on simple chemical and biochemical data. It is the latter area that the SCM may be most useful, particularly if (as in pesticide studies) there are a full range of required tests. The SCM should demonstrate where to look and how to look for problems in field trials, would determine the types of plant uptake studies to be performed, and should indicate if secondary effects (reduced yield, phytotoxicity, soil community effects, etc.) need to be evaluated in depth. Obviously, any test system which could reduce the cost of subsequent experiments

at a lesser cost should be valuable. The purpose of the SCM test is not to label a chemical "bad", but rather to provide a means of discriminating between levels of concern about problems of fate and effect. Depending on the structure of a hazard evaluation system in which the SCM test results are used, these results could justify further testing or permit by-passing such testing.

REFERENCES

Bostick, W. D. and B. S. Ausmus. (In Press). Methodologies for determination of adenosine phosphates. Anal. Biochem.

Chiou, C. 1979. Oregon State University, personal communication.

Cole, L. K., R. L. Metcalf, and J. R. Sanborn. 1976. Environmental fate of insecticides in terrestrial model ecosystems. Intern. J. Environ. Studies 10:7-14.

Draggon, S. 1976. The microcosm as a tool for estimation of environmental transport of toxic materials. Intern. J. Environ. Studies. 10:76-70.

Draggon, S. 1979. The effects of substrate type and arsenic dosage level on arsenic behavior in grassland microcosms, Part I: Preliminary results on ^{71}As transport. In: Terrestrial Microcosms and Environmental Chemistry (J. M. Witt and J. Gillett, eds.). Corvallis, Oregon. National Science Foundation NSF/RA 79-0026, Washington, D.C. pp 102-110.

EPA. 1977. Soil core microcosm test. IERL-RIP Procedures Manual: Level I Environmental Assessment Biological Tests for Pilot Studies. EPA-600/7-77-043.

Gile, J. D. and J. W. Gillett. 1979a. Fate of ^{14}C dieldrin in a simulated terrestrial ecosystem. Arch. Environ. Contam. Toxicol. 8:107.

Gile, J. D. and J. W. Gillett. 1979b. The Fate of Selected Fungicides in a Terrestrial Laboratory Ecosystem. Submitted to J. Agric. Food Chem. June 1979.

Gillett, J. W. and J. M. Witt (ed.). 1979. Appendix B: Soil Core Microcosm Screening Protocol. In: The Proceedings of the Workshop on Terrestrial Microcosms, June 15-17, 1977, Corvallis, Oregon. National Science Foundation NSF/RA 790034. Washington, D.C.

Gillett, J. W., and J. D. Gile. 1976. Pesticide fate in a terrestrial laboratory ccosystem. Intern. J. Environ. Studies 10:15-22.

Jackson, D. R., C. D. Washburne and B. S. Ausmus. 1977. Loss of Ca and NO_3^{-1} from terrestrial microcosms as an indicator of soil pollution. Water, Air Soil Pollut. 8:271.

Jackson, D. R. and J. M. Hall. 1978. Extraction of nutrients from intact soil cores to assess the impact of chemical toxicants on soil. Pedobiologia 18:272-9.

Johnson, E. L. 1978. Proposed guidelines for registering pesticides in the United States. Federal Register 43:(132)29696-29741.

Lassiter, R. 1979. E.P.A. Environmental Research Laboratory, Athens, Ga, personal communication.

Lee, J. J. and D. E. Weber. 1976. A study of the effects of acid rain on model forest ecosystems: In: Proceedings of the Air Pollution Control Association Annual Meeting.

Lichtenstein, E. P., T. W. Fuhremann and K. R. Schulz. 1974. Translocation and metabolism of ^{14}C-phorate as affected by percolating water in a model soil-plant ecosystem. J. Agric. Food Chem. 22:99-96.

Lighthart, B., and H. Bond. 1976. Design and preliminary results from soil/litter microcosms. Intern. J. Environ. Studies 10:51-58.

Metcalf, R. L., L. K. Cole, S. G. Wood, D. J. Mandel and M. L. Milbrath. 1979. Design and evaluation of a terrestrial model ecosystem for evaluation of substitute pesticide chemicals. EPA-600/3-79-004. U.S. Environmental Protection Agency, Corvallis, OR. 299 pp.

Muir, W. 1979. Toxic Substances Control Act. Premanufacturing Testing of New Chemical Substances. Federal Register. 49:(53)16240-16292.

Nash, R. G., M. L. Beall, Jr., and W. G. Harris. 1977. Toxaphene and 1.1.1-Trichloro-2,2-bis [p-chlorophenyl] ethane (DDT) losses from cotton in an agroecosystem chamber. J. Agric. Food Chem. 25:336-341.

O'Neill, R. V., B. S. Ausmus, D. R. Jackson, R. I. Van Hooks, P. Van Voris, C. D. Washburne and A. P. Watson. 1977. Monitoring terrestrial ecosystems by analysis of nutrient export. Water, Air Soil Pollut. 8:271-277.

Rand, M. C., A. E. Greenberry and M. J. Taras (ed.). 1975. Standard Methods for the Examination of Waste and Wastewater. 14th ed.

Snedecor, G. W. and W. G. Cochran. 1969. Statistical Methods (6th ed.). The Iowa State University Press.

Spencer, E. Y. 1973. Guide to Chemicals Used in Crop Protection (6th ed.). University of Western Ontario. Publication 1093. 542 p.

Spencer, W. F. and M. M. Cliath. 1969. Vapor density of dieldrin. Environ. Sci. Technol. 3:670.

Van Voris, P., R. V. O'Neill, H. H. Shugart, W. R. Emanuel. 1978. Functional Complexity and Ecosystem Stability: An Experimental Approach. ORNL/TM-6199. 120 p.

Witt, J. M. and J. W. Gillett (ed.). 1979. "Terrestrial Microcosms and Environmental Chemistry," The Proceedings of a Symposium on June 13-14, 1977, Corvallis, Oregon. National Science Foundation, NSF/RA 79-0026. Washington, D.C. 147 pp.

APPENDIX A

SOIL CORE MICROCOSM SCREENING PROTOCOL

The soil microcosm test should yield a reasonably rapid yes/no answer to questions of short-term contaminant effects. Since terrestrial accumulation sites and remineralization processes are predominantly within soil, intact soil microcosms excised from representative target systems are used as test units. The description which follows is taken from the work of Ausmus et al. (1977, as reported in Gillett and Witt, 1979).

Obtain soil cores 5- cm diameter x 10-cm depth from representative terrestrial ecosystems. Encase the cores in a 1- to 3-mil-thick teflon with 1- to 3-mil-thick shrinkable polyvinyl chloride and gently heat shrink until a tight bond with the core (minimum boundary flow) is achieved. Leave enough lining above the soil surface to use gaseous export traps if necessary. Mount on glass funnels in test tubes. Cover sides with opaque wrappings to negate abnormal algal growth. Place in environmental chamber under as near the field conditions as possible.

Equilibrate 3 weeks, if possible. Leach with rainwater or reconstituted water (known water chemistry) 2 to 3 times (enough to obtain 20 to 30 ml/date during equilibration). Determine Ca and dissolved organic carbon (DOC) concentrations in these samples. If possible, use alkali traps to determine daily CO_2, flux 3 to 10 days during equilibration. Use these data to discard dissimilar replicate soil cores and establish behavior of individual replicates.

Experimental design is preferably a randomized complete block and, if possible, factorial treatment arrangement of dosages with a minimum of three cores per dose per terrestrial ecosystem tested. Randomized incomplete block designs can be used to test a large number of contaminants simultaneously. Dosages of a wide range should be used for this phase to maximize the clarity of dose-dependent observations.

Add the test contaminant to the surface of the cores in a carrier, such as soil (taken from replicate cores to those used as experimental units). Dosages might be 0, 10, 100, 1000 ppm, for example, based on core weights. Total amendment to all cores should be equivalent so that, for example, a control replicate would receive carrier (such as soil) equivalent to the carrier plus contaminant received by a treatment dose. Contaminant and carrier should be well mixed prior to uniform deposition onto the core surface.

Set gas traps to monitor CO_2 recovery. Collect traps and titrate with 0.1 M HCl at 24-hour intervals, if possible. The more frequent the

measurement, the more complete data analysis can be, however, diurnal rhythms make daily observation the minimum useful time period. Weekly measures are practically useless.

After a week, add sufficient rainwater or reconstituted water (known water chemistry), to collect approximately 20 ml of leachate per core. Analyze Ca and DOC concentration. Determine contaminant concentration using standard chemical techniques. On days 14 and 21 repeat leaching.

Perform intact extraction techniques for pools of nutrients left within the core to estimate mass balances for microcosm units (Jackson and Hall, 1978). This technique is the addition of 200 ml of 1.0 M KCl or $NaHCO_3$ to cores and measurement of Ca, DOC, and the contaminant, respectively, in the leachate.

Biotic analyses could also be conducted. Before extraction, core samples 1 cm in diameter should be removed from the soil microcosms using a cork borer. The hole should be filled by a glass rod of the approximate size when extraction for contaminant and nutrients is to be performed.

Biotic analysis could use the ATP assay or adenylate energy charge (Bostick and Ausmus, in press) which would allow relative microbial pools to be compared across treatment levels. The procedure for ATP analysis is to add 1 g of soil (wet weight) to 6 ml of pH 7.4 TRIS buffer with 0.06 g ethylenediaminetetraacecetic acid (EDTA). Vortex briefly. Add 3 ml of chloroform. Vortex again. Sonify in ice water 2 to 5 minutes. Centrifuge (preferably at low temperature) at 100 x G for 2 to 10 minutes. Transfer buffer to new tube. Add 3 ml of CCL_4. Recentrifuge briefly. Sample buffer phase. Assay at 340 nm using standard hexokinase reaction or a fluorescence spectophotometer (if sensitivity greater than 0.5 ppm is required).

Divide cores into 1-cm depths. Within each depth measure the amount of contaminant by radiosotopic or standard chemical techniques. This is an optional step, useful if the distribution of the contaminant is to be estimated.

Both monitoring data and harvest data will be available on nutrient processes and microbial activity.

Monitoring Results

Calculate the total export of Ca, DOC, and contaminant for each microcosm by date using concentrations detected and volumes of leachate collected. Calculate mean export (with standard error) by treatment dose for each contaminant. These data may be expressed as cumulative export and plotted as a function of time. CO_2 efflux or other gaseous export data can be similarly summarized and presented. Statistical comparison can be made by covariance to determine the effect of treatment on export of nutrients and contaminant. Previous studies show that CO_2 efflux and nutrient export often increase as a function of dose. However, Ca and CO_2 release may be inhibited by some toxic

compounds or these may show biphasic behavior, depending on the concentration of toxicant. Transport of the contaminant is usually greater with increasing dose.

Harvest Results

Calculate the extractable Ca, DOC, or contaminant based on concentrations measured multiplied by extracted volumes. Calculate means (with standard errors) across replicates for each treatment dose. Use standard analysis of variance of Duncan's Range Test to determine differences due to treatment. Biotic data may be summarized as ATP per gram of soil by 1-cm depth intervals for each dosage. ATP concentrations may be increased or decreased by the contaminant, depending on the specific microbial population impacted.

Soil Core Microcosm Experiment I

Purpose

The purpose of this experiment is to yield data reflecting the fate and effects of ^{14}C dieldrin, parathion and 2,4,5-T on a soil ecosystem and to determine the suitability of the soil core microcosm as a screening tool.

Experimental Approach

Select a 1 sq meter area in the field behind the CERL trailers and clip grass down to surface: this will serve as the source for the soil cores. There will be 15 cores per treatment for a total of 60 including control treatment.

Obtain soil cores 5 cm dia x 10 cm depth and place each in a sealed plastic bag for transport to lab. Once in the laboratory remove cores and clip above ground vegetation down to soil surface. Fit each core with polyethylene base and encase in Teflon and shrinkable PVC and gently heat shrink until a tight bond with the core is achieved. Leave approximately 5 cm of tubing above soil surface to use with gaseous export traps if necessary. Mount on glass funnel (seal interface of core and funnel with silicon rubber) in 250 ml flask. Place in incubator adjusted for field conditions or in greenhouse.

Equilibrate cores for 28 days. Leach with std ref water (i.e. standard ref. rainwater) on days 7, 14, 21 and 28 (enough to obtain 20 to 30 ml/date). NH_3; NO_3^{-1} and PO_4^{-3} will be determined via Technicon Auto Analyzer by Northrop personnel. Northrop personnel will prepare and deliver approx. 20 ml of leachate to LASS for Ca^{+2} and DOC analysis. Certified standards and blanks will be run with all samples. The certified standards and methods for preparation are available from W. Griffis (LASS). Standards and blanks should be verified routinely. Exceptional care must be exercised in the preparation of all samples due to the high probability of contamination from both the human body and local environment. Also use KOH traps to determine CO_2 efflux for 24 hr on Monday and Thursday.

On day 28 (after leaching) apply pesticides at the rate of 1 lb/acre (15 cores/treatment). Use standard carriers for dieldrin, parathion and 2,4,5-T; treat control with dieldrin carrier. Use a pipette to apply material evenly to surface of core; rinse pipette and apply rinse to core.

Use 0.2 N KOH traps for CO_2 efflux, collect traps and titrate with 0.1 M HCl for 24 hrs. on every Monday and Thursday.

On day 35 add sufficient std. ref. water to collect 30 ml of leachate/ core. Analyze for same parameters as during equilibration period plus pesticide content, repeat process on days 42 and 49 for all of the above parameters.

On days 28, 35, 42 and 49 remove 1 core from each treatment at random, subdivide into three layers, 0-2 cm, 2-5 cm and 5-10 cm. From top layer remove all biotic material (plant root and shoot and visible animal species); weigh and analyze for pesticide. Perform intact extraction of all 3 levels for pools of nutrients left within the core. Use either 1 M KCl or $NaHCO_3$ for extraction. After nutrient extraction treat soil samples by prescribed methods for ^{14}C analysis. Nutrient extraction should also be analyzed for ^{14}C. On day 56, terminate cores and perform intact extraction process and ^{14}C analysis.

For 3 treatments + control there will be approximately 380 leachate samples, 960 CO_2 samples and 180 (60 x 3 subsamples) destructive samples.

Soil Core Microcosm Experiment II

Purpose

The purpose of this experiment is to yield data reflecting the fate and effects of ^{14}C labeled HCB on a soil ecosystem, as well as continuing evaluation of the soil core system as a potential screen for TSCA.

Experimental Approach

Select a 5-sq meter area at Schmidt Farm, free of agricultural chemicals. This will serve as the source for the soil cores. There will be 21 cores per treatment level (3 levels: 0.25, 0.5, and 1 lb/acre) for a total of 84 including control treatment.

Obtain soil cores 5-cm dia x 8-cm minimum depth and place in a sealed plastic bag for transport to lab. Once in the laboratory remove cores and fit each with a polyethylene base and encase in shrinkable PVC. Gently heat shrink until a tight bond with the core is achieved. Leave approximately 5 cm of tubing above soil surface to use with gaseous export traps if necessary. Mount on glass funnel (seal interface of core and funnel with silicon rubber) in 250 ml flask. Place in growth chamber adjusted for field conditions.

Equilibrate cores for 28 days. Leach with std. ref. water (i.e. standard ref. rainwater) on days 7, 14, 21 and 28 (enough to obtain 30 ml/date). Determine NO_3^{-1}, NH_3 and PO_4^{-3} concentrations of leachate via Technicon Auto Analyzer by Northrop personnel. Northrop personnel will prepare and deliver approx. 20 ml of leachate to LASS for Ca^{+2} and DOC analysis.

Use 0.2 N KOH traps for CO_2 efflux, collect traps and titrate from 5 cores/treatment with 0.1 M HCl for 24 hrs on every Monday. Certified standards and blanks will be run with all samples. The certified standards and methods for preparation are available from W. Griffis (LASS). Standards and blanks should be verified routinely. Exceptional care must be exercised in the preparation of all samples due to the high probability of contamination from both the human body and local environment.

On day 28 (after leaching) apply HCB at the prescribed rate (15-18 cores/treatment). Use standard carrier for HCB, treat 1/2 control with carrier and balance with distilled H_2O. Use a pipette to apply material evenly to surface of core, rinse pipette and apply rinse to core.

On day 35 add sufficient std. ref. water to collect 30 ml of leachate/core. Analyze for same parameters as during equilibration period plus pesticide content, repeat process on days 42 and 49 for all of the above parameters.

On days 28, 35, 42 and 49 remove 1 core from each treatment at random, subdivide into three layers, 0-2 cm, 2-5 cm and 5-8 cm. From top layer remove

all biotic material (plant root and shoot and visible animal species); weigh and analyze for pesticide. Perform intact extraction of all 3 levels for pools of nutrients left within the core. Use either 1 M KCl or $NaHCO_3$ for extraction. After nutrient extraction treat soil samples by prescribed methods for ^{14}C analysis. Nutrient extraction should be analyzed for ^{14}C. On day 56, terminate cores and perform intact extraction process and ^{14}C analysis.

For 3 treatments + control there will be approximately 480 leachate samples, 140 CO_2 samples and 160 (54 x 3 subsamples) destructive samples.

APPENDIX B

Standard Reference Rainwater Formula (Lee and Weber, 1976).

The standard "rain" solution was made from deionized distilled water, $CuSO_4$, $MgCl_2$, KCl, NaCl and NH_4NO_3 to give the following compositon.

Ca^{+2}	0.22 mg/l
NH_4^+	0.22 mg/l
Na^+	0.11 mg/l
K^+	0.06 mg/l
Mg^{+2}	0.08 mg/l
SO_4^{-2}	0.48 mg/l
NO_3^-	0.74 mg/l
Cl^-	0.53 mg/l

APPENDIX C

QUALITY CONTROL IN PROCEDURES FOR SOIL CORE MICROCOSMS

Treatment

The soil core microcosms are treated with mixtures of labeled and unlabeled pesticides. The specific activity of the labeled material is known from the manufacturer's literature. It is combined with the unlabeled material to give the desired specific activity for a given application. The purity of the labeled material is determined by FID and ECD gas chromatography, thin-layer chromatography in three different solvent systems and autoradiography. The purity of the unlabeled material is determined by FID and ECD gas chromatography only. The combined pesticide (in xylene solution) is emulsified with water. The specific activity is checked a final time and the emulsion is applied to the plant material (foliar application) with a pipette.

Each week 40 ml of "rain" is poured onto the tops of the cores. On the average this gives about 30 ml leachate with which to work. Those that leach in less than 10 min or greater than 12 hours are discarded. The leachate volume is measured and the leachate stored in vials and refrigerated. The flasks are rinsed with deionized distilled water and the SCM's reassembled.

Quality assurance begins with proper handling of the samples to be analyzed. The leachates are not chemically stabilized so they are stored in the refrigerator to prevent degradation. This is followed by careful transfer of the leachates and KCl extracts to AA II sample cups. The complete set of standards is run at least twice each day as well as replicates of samples and EPA quality control samples. Periodically, samples (including standards and QC samples) are sent through the LASS (Laboratory Analytical Support Staff) Autoanalyzer system.

The basic information concerning set-up, adjustment, operation and maintenance of the AA II system can be found in the manuals supplied by Technicon. Briefly, the following precautions should be taken in the set-up and operation of the AA II system.

Setup:

1. Are the manifolds set up properly? (This includes proper pump tube sizes and transmission lines connected to the correct reagent bottles.)

2. Are the reagents freshly made and of the correct composition?

3. Is the heating bath on? (If required.)

4. Are the correct filter and cell installed in the colorimeter?

5. Is the colorimeter on?

Operation:

1. Is the proportioning pump on?

2. Are the air bubbles evenly spaced?

3. Is the sampling rate correct?

4. Is the sampler on?

5. Is the recorder on?

6. Is the baseline smooth and stable?

7. Is the digital printer on?

Results

1. Do the standards give peak heights in the proper range and are the results linear in that range?

2. Do the QC samples give proper values?

Maintenance

1. The pump tubes should be changed each week.

2. The proportioning pump should be lubricated as per the Technicon manual.

Pesticide Residue Analysis.

The following fractions of the SCM's are analyzed separately for pesticide residues: leachates, soil, plant material, polyvinylchloride and masking tape. Liquid scintillation spectrometry is used to trace the residual material in the samples. Each type of sample is analyzed using a specific procedure. The activity of the resulting fractions is then determined. Those that are found to have sufficient activity are analyzed further by thin-layer chromatography and autoradiography. The residues found in this manner can be classified in one of the following groups: intact pesticide, non-polar metabolite, polar metabolite and nonextractable (bound) residue. From the data acquired the biodegradability index and ecological magnification for each type of sample can be determined.

The following brief list of precautions in the analysis for pesticide residues will assist in the maintenance of quality control.

Analysis: (intact pesticide, polar and non-polar metabolites)

1. Is it the proper procedure for the type of sample?

2. Is there complete transfer of material?

3. Are the measurements of masses and volumes accurate?

4. Is all data written down promptly and correctly?

Sample Oxidation: (non-extractable bound residue)

1. Start-up of Packard 306 Sample Oxidizer:

   a. Has the waste jug been emptied?

   b. Is the methanol gas trap full?

   c. Are the reagent reservoirs full?

   d. Do the N_2 and O_2 cylinders have sufficient pressure?

   e. Is the distilled water reservoir full?

   f. Gas cylinders on?

   g. Power on?

   h. Is the distilled water switch on PRESSURE?

2. Operation:

   a. Do the reagent dispensers have the proper settings? Are they working properly?

   b. Does the timer have the proper setting? Is it working properly?

   c. Is the ignition basket in good condition? (Clean? Coils properly spaced?)

   d. Are the samples burning properly? (Sufficient oxygen and/or burn time? High voltage switch stuck? Are there leaks in the system? Leaking 4-way valve? Pneumatic mechanisms operating properly?)

3. Shutdown:

   a. Is the power off?

   b. Is the distilled water switch on VENT?

c. Gas cylinders off?

d. Is the instrument cleaned up?

4. Results:

a. Are the AES ratios consistent and in the proper range?

b. Are the values for samples and spikes reasonable?

c. Is the recovery good?

d. Is there low carryover?

Sample Counting:

1. Set-up of the Packard 3385 Liquid Scintillation Spectrometer:

a. Are the samples set up properly?

b. Is there a blank of the same cocktail in each group of samples?

c. Is the channel set for the proper isotope?

d. Are the preset count and preset time of their correct values for the results desired?

e. Is the desired information printed out?

2. Results:

a. Are the AES values consistent with the type of cocktail being used and the expected amount of quenching?

b. Are the activity values reasonable?

c. Is the background value reasonable?

Thin-Layer Choromtography and Autoradiography: The following is a brief outline of the procedures used to analyze extracts by thin-layer chromatography and auto-radiography and precautions taken to assure good results.

1. Thin-Layer Chromatography

Precautions	Sample	Procedure
		1. The volume is measured out which will give 10^4 dpm or the entire sample if the total activity is between 10^3 and 10^4 dpm.
Correct volume?		

2. The volume is reduced (if necessary) to 20 ml on the rotary evaporator.

100% transfer of material?

3. The volume is reduced further (if necessary) to 2 ml with a stream of nitrogen.

4. If necessary, the sample is filtered through a small column of anhydrous Na_2SO_4.

Material left on column?

5. The sample is spotted.

100% transfer of material?

6. 10^4 dpm of the reference standard is spotted.

7. The plate is developed.

Proper solvent system used?

2. Autoradiography

Precautions	Developed Plates	Procedure
Correct orientation in box?		1. The plates are placed in box with x-ray film (stacked alternately) and allowed to sit 4 weeks.
Chemicals in good condition?		2. After 4 weeks the film is developed.
Proper temperature used?		3. A trace is made of all darkened spots on the film. The spots are numbered and their distances from the origin measured.
Correct film-paper orientation?		4. All identified spots are removed from the plates and placed in scintillation vials.
Correct paper-plate orientation?		5. Cocktail is added to the vials and the samples are counted.
Accurate spot removal?		

Overall, approximately 25-30% of analysis time is spent on some aspect of quality assurance.

END

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